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SPOTLIGHT

Riding up the escaLEC-TOR for valvular health

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Endothelial-lined valves assure unidirectional flow in the lymphatic system. In this issue, Saygili Demir et al. (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202207049) demonstrate how continuous repair of these valves occur, beginning with mTOR-activated cell replication in valve sinuses, and followed by cell migration to cover the valve surface.

Vertebrates have two vascular systems, not just one. In the "second" system, lymphatics vessels transport interstitial fluid from nearly all tissues back into the venous system. Interstitial fluid is initially transported by convection into open-ended lymphatic capillaries, and subsequently flows to collecting lymphatics and ultimately the thoracic duct to return to the bloodstream. Interstitial flow is 100–500 slower than that of blood, and, in contrast to the blood system, the lymphatic system lacks a central pump. The forces of convection are thus generally low in collecting vessels, and unidirectional transport is sustained by valves, eponymously named after Jan Swammerdam (1637–1680), who used injections of suet, wax, and dyes into cadavers to first describe lymphatic valves almost 400 yr ago.

Lymphatic valves are bi-leaflet and have been described as forming a funnel-like shape, tapering to a small downstream opening that closes when forces favor retrograde flow (Fig. 1 A; 1). The valves are optimized for low-flow and viscous states, and the closing of the valves relies almost entirely on the unique viscous pressure generated within the sinuses between the leaflets and the vascular wall. Like the rest of the lymphatic system, the valve leaflets are covered by lymphatic endothelial cells (LECs). These LECs are thus exposed to substantial shear stress, and prone to damage and loss. Not surprisingly, the formation and maintenance of lymphatic valves depends on flow and shear stress (2). The question thus presents: How is homeostatic replenishment of these LECs maintained?

Using an elegant series of experiments with sophisticated imaging and genetically modified mice, Saygili Demir et al. (3) now describe a compelling model for how LECs proliferate and regenerate in adult lymphatic vasculature. The initial and striking observation was that the highest rate of LEC turnover in adult lymphatics occurs within valve sinuses, in sharp contrast to the highest LEC turnover in capillaries during development. The authors go on to show that: (i) these sinus-born LECs migrate along the valve leaflet to the terminal valve opening, and (ii) blocking the proliferation of these LECs causes valvular rarefaction. From these observations, the authors propose an "escalator" model, whereby valve leaflets exposed to high shear stress are prone to damage and loss of LECs, which are then regenerated by LECs generated in the sinuses, where the authors propose active division is safer because there is less physical movement (Fig. 1 B).

What activates LEC proliferation in the sinuses? The authors use neutralizing antibodies to show that it is not the VEGF-A/VEGFR-2 or VEGF-C/VEGFR-3 pathways, despite their indispensable contribution to LEC proliferation during development, underscoring the differences between development and adult homeostasis. Instead, the authors discovered that the signal for proliferation is shear stress and involves the mechanistic target of rapamycin (mTOR) pathway. mTOR is a central sensor and effector of cellular metabolism and can powerfully regulate cellular growth (4). mTOR activation is widely associated with hyperplasia and neoplasia. Previous work had shown that mTOR activity was necessary for lymphangiogenesis and for maintaining pre-established lymphangiectasias, in a VEGR2/3-independent fashion (5), and mTOR inhibitors have shown clinical efficacy against lymphangioleiomyomatosis (6). It is perhaps not surprising, then, that the authors found that mTOR activity was necessary and sufficient for LEC proliferation and valve formation. But the authors expand on this observation, elegantly demonstrating that the trigger for mTOR activation is shear stress, more specifically low shear stress (LSS), analogous to that found in the valve sinuses, where peak mTOR activation and peak proliferation are found. The observations thus convincingly build upon the "escaLEC-TOR" model, whereby LSS in the sinuses activates mTOR to promote LEC proliferation and migration up the valve leaflets (Fig. 1 B).

Like most models, it explains observed data but also poses additional questions. For example, the model addresses homeostasis of LECs lining the leeward side of the valves, but what of the windward side? Surely the damaging sheer stress is equally if not more damaging on that side. Documenting which portions of the valves undergo apoptosis or other cell death would be informative on this point. It may also be that LECs proliferating in

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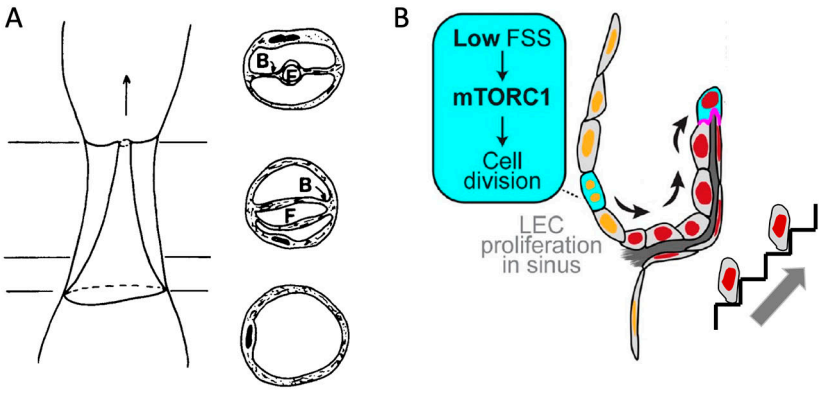

Figure 1. **Riding up the escaLEC-TOR for valvular health. (A)** Funnel-like arrangement of lymphatic valves. Modified from Schmid-Schönbein, 1990 (1). F: funnel. B: buttresses attaching valve leaflets to lymphatic wall. **(B)** Escalator model whereby low flow shear stress (FSS) in the sinuses activates mTOR to promote LEC proliferation and migration up the valve leaflets. Modified from Saygili Demir et al., 2023 (3).

the sinus migrate further into the lymphangion, contributing to homeostasis there. Edu labeling at 4 wk by the authors suggests this possibility, which is worth further investigation. It will also be interesting to investigate these pathways beyond gut lymphatics, in which the current study is focused.

How does LSS activate mTOR? LSS has been reported to activate mTORC1 in endothelial cells, but in that case too the upstream sensor remains uncertain (7). Akt1, an established activator of mTOR, is a likely candidate. Akt1$^{-/-}$ mice reveal substantial loss of valves in collecting lymphatics (8), and Akt1 activation promotes valve formation (9). And loss of Foxo1, which Akt1 antagonizes, has the opposite effect, promoting valve formation (10,11). These latter studies are interesting, because the regulation of Foxo1 by Akt1 is a separate arm from the regulation of mTORC1, suggesting that Akt1 could be an upstream hub for multiple inputs to modulate adult lymphatic valve formation and maintenance. But what cell surface sensor communicates LSS to Akt1 or mTOR remains uncertain. Equally interesting and uncertain are the downstream effectors of mTOR, i.e., how, in the context of LECs, does mTORC1 activation promote cell proliferation and migration? A metabolic component seems likely.

Another interesting question is whether this homeostatic mechanism is constitutive or induced by damage, i.e., is the escalator constantly running, or activated by damage? If the latter, the additional question of how damage would be communicated at a distance to the sinus LECs is raised. In the blood vascular tree, vasodilatory signals are propagated upstream to arterioles for blood flow control via multiple mechanisms, most prominently including electrotonic impulses transmitted via gap junctions (12). The same may be true here.

Finally, it is interesting to think of these findings in the context of various clinical settings. For example, lymphedema tarda, a late-presenting form of primary lymphedema syndromes and often triggered by infection, is notably marked by the absence of valves. Would mTORC1 activators help in these patients? Does infection worsen phenotypes in the authors' mouse models? Do the findings also help with thinking about therapy to treat breast cancer–related upper limb lymphedema? Ongoing efforts with lymph node transfer supplemented with VEGF-C–containing vectors are promising but likely need improvement (13). Finally, the work may explain a longstanding observation that lymphedema frequently complicates treatment with rapamycin for immunosuppression (14), although valvular insufficiency has not specifically been investigated in these patients. It will be interesting to see what happens to valves with long-term mTORC1 inhibition in mice as in humans, especially because such long-term treatment has raised much interest as potentially lifespan prolonging.

Acknowledgments

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